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Comparative Effect of Pretreatment with Phenobarbital, Aroclor 1254, and beta-Naphthoflavone on the Metabolism of Lindane

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16. ABSTRACT

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Comparative Effect of Pretreatment with Phenobarbital, Aroclor 1254, and β-Naphthoflavone on the Metabolism of Lindane[1,2]

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An attempt was made to distinguish different patterns of microsomal enzyme induction by phenobarbital, β-naphthoflavone, and Aroclor 1254 on the biotransformation and excretion of the organochlorine insecticide lindane. Treated groups of six weanling female Sprague–Dawley rats, individually housed in metabolism cages, received diets containing either 500 ppm Aroclor 1254, 356 ppm phenobarbital, or 418 ppm β-naphthoflavone. After 1 week all animals, except one group of controls, were dosed p.o. with 1.89 mg lindane (containing 1.63 μCi [U-^{14}C]lindane). Twenty-four hours later the rats were sacrificed and urine, feces, expired air, and tissue samples were taken for analysis of radioactivity. Hepatic cytochrome *P*-450 content, microsomal phospholipid content, and the enzyme activity involved in the dehydrogenation of lindane, the dechlorination of lindane, and the hydroxylation of the intermediate hexachlorocyclohexene were determined *in vitro*. Moreover, the effect of pretreatment on the excretion of radioactivity and the distribution of eight lindane metabolites was examined. Even though the rate of lindane metabolism was unchanged by the β-naphthoflavone pretreatment, results of the study indicated that all three pretreatments significantly altered lindane metabolism. The pretreatments differed from one another in that they selectively altered specific metabolic pathways.

INTRODUCTION

Induction of hepatic microsomal enzymes can potentially intensify the toxicity of pesticides and other environmental chemicals by activating the conversion of these compounds to chemically reactive metabolites that mediate carcinogenesis, mutagenesis, cellular necrosis, hypersensitivity reactions, methemoglobinemia, hemolytic anemia, blood dyscrasias, and fetotoxicity (1). Multiple species of the terminal electron transport oxidase, cytochrome *P*-450, have been distinguished and these species have different catalytic properties and substrate specificities (2, 3). Thus preferential induction of select species could be responsible for the stimulation of specific metabolic pathways and an increased conversion of toxicants to reactive metabolites via these routes. In fact, a minimum of 12 different induction patterns of hepatic microsomal polypeptides, in the molecular weight range of 47–56 kdalton, have been observed after rats were treated with various drugs and chemicals (4). Specific qualitative changes in drug-metabolizing enzyme activity were recently reported after treatment of rats with a variety of pesticides (5), and there is evidence that the *in vivo* biotransformation of pesticides is selectively altered after pretreatment with various enzyme-inducing agents (6–10). Furthermore, hepatic microsomal enzymes are located in the endoplasmic reticulum which is composed of from 30 to 55% lipid (11). Microsomal phospholipids are not only structural and storage components but also play a role in the regulation of enzyme activity and membrane permeability (12–15). Thus diversity in the in-

[1] This paper was presented in part at the 76th Annual Meeting of the North Carolina Academy of Science, Greensboro, N.C. in March 1979.

[2] This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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duction of hepatic microsomal enzymes may also be related to changes in the phospholipid structure and the functional state of the enzymes.

Recently, it has been suggested that there may be a carcinogenic risk to man associated with the use of the insecticide lindane (γ-hexachlorocyclohexane) in pharmaceutical preparations,[3] on livestock, and in various agricultural applications (16). Since most chemical carcinogens in the environment require metabolic activation (17–20), it is important to determine how the normal biotransformation of this pesticide is altered after induction of hepatic microsomal enzymes. The purpose of this study was to determine the effect of pretreatment for 1 week with either phenobarbital, β-naphthoflavone, or Aroclor 1254 on the multiple pathways involved in the biotransformation of lindane by rats.

MATERIALS AND METHODS

Thirty weanling female Sprague–Dawley rats, individually housed in metabolism cages were randomly assigned to one of five treatment groups. Two of these groups were fed unadulterated Purina lab chow. Of the remaining three groups, one was fed Purina lab chow containing 500 ppm Aroclor 1254 (Monsanto Chemicals, St. Louis, Mo.), another was fed Purina lab chow with 356 ppm crystalline phenobarbital (Sigma Chemical Co., St. Louis, Mo.), and the third group received Purina lab chow containing 418 ppm β-naphthoflavone (Aldrich Chemical Co., Inc., Milwaukee, Wisc.). Diets were prepared as described elsewhere (21). Based on average food consumption and the level of Aroclor 1254 used for induction in the Ames assay (22) these concentrations were an attempt to deliver equimolar, maximally effective quantities of the inducing agents to the animals. After 1 week all animals, except six in one control group, were dosed p.o. with 1.89 mg lindane (containing 1.63 μCi [U-^{14}C]lindane). The [U-^{14}C]lindane (sp act 48 mCi/mmol, radiochemical purity 98%) was obtained from Amersham, Arlington Heights, Illinois. The unlabeled lindane (>99% γ-hexachlorocyclohexane) was obtained from the E.P.A. Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, North Carolina. After treatment with lindane, the rats were transferred to animal containment chambers (Plas-Labs, Lansing, Mich.) and expired air was passed through Carbosorb II (Packard, Downers Grove, Ill.) for CO_2 collection (23). Fasted for the next 24 hr, the rats were then sacrificed with samples of urine, feces, expired air, fat, liver, and kidney taken for analysis of radioactivity.

Liver, kidney, fat, and feces samples were subjected to combustion in a Packard Tri-Carb Model 306 sample oxidizer and the radioactivity was determined in a Packard Tri-Carb Model 3380 liquid scintillation spectrometer employing a quench correction curve. Hepatic content of cytochrome *P*-450 (24–25), and the enzyme activities involved in the dehydrogenation of lindane, the dechlorination of lindane, and the hydroxylation of hexachlorocyclohexene (synthesized by photochlorination of *o*-dichlorobenzene (26)) were determined (27). Lipids were extracted using a modification (28) of the Bligh and Dyer method (29). They were assayed by the method of Fewster (30) modified as follows: tlc plates were sprayed with 6% cupric acetate in 15% phosphoric acid and were heated at 160°C for 15 min.

Individual 24-hr urine samples were collected, extracted, and the extracts analyzed on a Tracor Model MT-220 gas chromatograph equipped with an electron capture detector and a Coulson electrolytic conductivity detector as previously described (31) and modified (32, 33).

Duncan's multiple range test (34) was used as an aid in the interpretation of the data from this study. Differences were con-

[3] Lindane is the primary active ingredient in products used to treat scabies and pediculosis (Kwell cream, lotion, and shampoo by Reed and Carnick and Gammene by Barnes-Hind Pharmaceuticals, Inc.).

sidered significant at $P < 0.05$ unless otherwise stated.

RESULTS

Aroclor 1254, at 500 ppm in the diet, caused a significant decrease in both food consumption and weight gain (Table 1). Accordingly, rats fed this diet received significantly less inducing agent than did animals treated with phenobarbital or β-naphthoflavone in the diet. In addition, the rats treated with Aroclor 1254 in their diet had significantly decreased body weights and kidney weights (Table 2). On the other hand, these animals had significantly larger livers and liver to body weight ratios than the other animals in the study (Table 2). All the animals dosed with lindane had significantly greater liver to body weight ratios than the untreated rats.

Within 24 hr after receiving [U-^{14}C]-lindane, rats pretreated with Aroclor 1254 or phenobarbital had excreted 82.8 and 77.8%, respectively, of the administered dose in urine, feces, and expired air (Table 3). Pretreatment with either Aroclor 1254 or phenobarbital resulted in nearly a fourfold increase in urinary radioactivity. In addition, Aroclor 1254 pretreatment produced a sevenfold increase in the radioactivity expired by control animals. In contrast, the radioactivity excreted by rats pretreated with β-naphthoflavone was not significantly different from that of the controls, and fecal radioactivity even fell to a level significantly less than that from rats pretreated with phenobarbital (Table 3).

Pretreatment with either Aroclor 1254 or phenobarbital resulted in significantly more radioactivity in all fractions than that excreted by control rats or by rats pretreated with β-naphthoflavone (Table 4). In addition, pretreatment with Aroclor 1254 altered the distribution of radioactivity with a significantly reduced proportion in the polar metabolite fraction and a twofold increase in the neutral fraction. The neutral fraction contains various chlorobenzene isomers, unconjugated alcohol isomers, free chlorophenols, and unaltered lindane. The polar fraction contains primarily mercapturic acid conjugates (35).

Both the controls and the β-naphthoflavone-treated rats stored about 5 times as much radioactivity in liver and kidney tissue and 25 times as much radioactivity in adipose tissue as did the animals fed diets containing Aroclor 1254 or phenobarbital (Table 5). The reduced storage of radioactivity in rats pretreated with Aroclor 1254 or phenobarbital was consistent with the significantly elevated excretion of radioactivity by these animals (Table 3).

TABLE 1
Effect of Inducers on Food Consumption and Weight Gain[a]

Treatment group[b]	Inducer intake (mmol/kg/day)	Food consumed (g/day)	Weight gain (g)
1. Controls	—	18.2 ± 0.5	18.0 ± 3.1
2. Lindane controls	—	18.0 ± 0.6	19.3 ± 2.8
3. Phenobarbital	0.23 ± 0.01	19.2 ± 0.8	27.7 ± 2.8
4. β-Naphthoflavone	0.22 ± 0.01	17.0 ± 0.5	22.2 ± 3.8
5. Aroclor 1254	0.19 ± 0.01	11.8 ± 0.8	−5.2 ± 4.2
Treatment comparisons	3,4 > 5	All > 5 3 > 4	All > 5

[a] Values are means ± SE.
[b] Treatment is described under Materials and Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

TABLE 2
Effects of Inducers on Body, Liver, Kidney Weight[a]

Treatment group[b]	Body weight (g)	Liver weight (g)	Kidney weight (g)	Liver weight/Body weight
1. Controls	116 ± 2	4.6 ± 0.2	1.30 ± 0.05	36.3 ± 2.8
2. Lindane controls	122 ± 4	5.7 ± 0.2	1.40 ± 0.04	47.0 ± 0.4
3. Phenobarbital	130 ± 5	6.5 ± 0.2	1.48 ± 0.06	50.6 ± 1.3
4. β-Naphthoflavone	120 ± 4	5.7 ± 0.3	1.40 ± 0.07	47.2 ± 1.6
5. Aroclor 1254	94 ± 6	9.6 ± 0.9	1.21 ± 0.07	102.0 ± 3.9
Treatment comparisons[c]	All > 5	5 > All 3 > 1	3,2,4 > 5	All > 1 5 > 3,4,2

[a] Values are means ± SE.
[b] Treatment is described under Materials and Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

The effect of induction on hepatic cytochrome *P*-450 content and some of the enzymes involved in lindane metabolism is presented in Fig. 1. Aroclor 1254 or phenobarbital pretreatment produced a significant increase in all the measured responses except the dechlorination of lindane where only Aroclor 1254 produced a significant increase in enzyme activity. The effect of β-naphthoflavone pretreatment on the enzyme activity was particularly interesting. While significantly stimulating the hydroxylation of hexachlorocyclohexene (HCCH) to 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL), β-naphthoflavone pretreatment impaired the enhanced dehydrogenase activity that was observed in the other three treatment groups dosed with lindane.

The effect of pretreatment with phenobarbital, β-naphthoflavone, or Aroclor 1254 on the relative proportions of eight lindane metabolites plus unaltered lindane in the urine is presented in Fig. 2. Pretreatment with all three chemicals significantly altered the metabolite excretion pattern from that of the controls. The proportion of the tetrachlorophenols 2,3,4,6-

TABLE 3
Effect of Pretreatment on Excretion of Radioactivity[a]

Treatment group[b]	Total excreted radioactivity		Radioactivity distribution (dpm × 10^{-3})		
	dpm × 10^{-3}	% Adm dose	Urine	Feces	Breath
1. Lindane control	787.4 ±130.1	22.1 ±3.65	714.0 ± 126.8	72.91 ± 13.69	0.424 ± 0.105
2. Phenobarbital	2776 ±108.1	77.8 ±3.02	2628 ± 123.1	147.3 ± 30.50	0.959 ± 0.111
3. β-Naphthoflavone	851.8 ±131.2	23.9 ±3.67	800.1 ± 116.9	51.18 ± 19.72	0.596 ± 0.132
4. Aroclor 1254	2953 ±184.2	82.8 ±5.18	2821 ± 193.4	128.4 ± 35.10	2.99 ± 0.384
Treatment comparisons[c]	4,2 > 3,1		4,2 > 3,1	2 > 3	4 > All

[a] Values are mean ± SE.
[b] Treatment is described under Materials and Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

TABLE 4

Effect of Induction on the Distribution of Radioactivity in the Urine[a]

	Radioactivity in urine							
	Neutral metabolites[c]		Free chlorophenols[d]		Conjugated metabolites[e]		Polar metabolites[f]	
Treatment group[b]	dpm × 10^{-3}	% Urine ^{14}C	dpm × 10^{-3}	% Urine ^{14}C	dpm × 10^{-3}	% Urine ^{14}C	dpm × 10^{-3}	% Urine ^{14}C
1. Lindane control	8.54 ± 1.16	1.33 ± 0.18	213.9 ± 32.4	30.8 ± 1.85	312 ± 70.3	42.3 ± 3.06	180 ± 28.9	25.6 ± 1.17
2. Phenobarbital	52.0 ± 9.17	1.97 ± 0.32	734 ± 65.6	28.1 ± 2.58	1270 ± 72.8	48.2 ± 1.53	576 ± 63.0	21.7 ± 1.89
3. β-Naphthoflavone	10.2 ± 2.16	1.26 ± 0.11	280 ± 67.5	33.4 ± 2.51	305 ± 22.0	40.0 ± 2.84	205 ± 33.4	25.4 ± 0.60
4. Aroclor 1254	112.0 ± 22.6	4.00 ± 0.84	904 ± 42.7	32.3 ± 1.03	1250 ± 108	43.9 ± 1.47	560 ± 51.6	19.7 ± 0.82
Treatment comparisons[g]	4,2 > 3,1	4 > All	4,2 > 3,1	N.S.	2,4 > 1,3	N.S.	2,4 > 3,1	1,3 > 4

[a] Values are means ± SE.
[b] Treatment is described under Materials and Methods.
[c] Radioactivity extracted with benzene from acid urine (pH = 2) which did not partition into 0.15 *N* NaOH.
[d] Radioactivity extracted with benzene from acid urine (pH = 2) which did partition into 0.15 *N* NaOH.
[e] Radioactivity extracted with benzene from hydrolyzed residual urine after the preliminary extraction.
[f] Radioactivity remaining in the residual hydrolyzed urine after extraction.
[g] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

TABLE 5
Effect of Induction on Tissue Radioactivity Content[a]

Treatment group[b]	Liver	Kidney	Adipose
1. Lindane control	7.13 ± 0.79	4.64 ± 1.10	338 ± 19.8
2. Phenobarbital	1.70 ± 0.34	0.93 ± 0.18	11.4 ± 1.99
3. β-Naphthoflavone	7.25 ± 0.57	6.87 ± 1.18	344 ± 51.7
4. Aroclor 1254	1.29 ± 0.26	1.00 ± 0.20	12.5 ± 5.00
Treatment comparisons[c]	3,1 > 2,4	3,1 > 4,2	3,1 > 4,2

[a] Values are mean dpm/mg tissue ± SE.
[b] Treatment is described under Materials and Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

and 2,3,4,5-TTCP[4] were significantly increased by pretreatment with either phenobarbital or Aroclor 1254. On the other hand, pretreatment with β-naphthoflavone also significantly altered the normal metabolite profile, despite the fact that there was no significant difference in radioactivity between β-naphthoflavone-pretreated rats and the controls. Rats dosed with β-naphthoflavone excreted significantly elevated proportions of 2,4,6-TCP, 2,3,5-TCP, and unaltered lindane. However, these animals excreted reduced proportions of 2,3,4,6-TTCP and PCCOL.

In addition to changes in the overall excretion pattern of the eight lindane metabolites, pretreatment with phenobarbital, β-naphthoflavone, or Aroclor 1254 changed the proportions excreted as sulfates, as glucuronides, or as unconjugated free metabolites (Tables 6–9). All three pretreatments significantly increased the proportion of the eight metabolites excreted as glucuronides from 69.9% of the total for the controls to about 85% for the treated animals (Table 6). Moreover, while having no significant effect on the percentage excreted free, the three pretreatments significantly reduced the proportion of the total excreted as sulfates. In rats pretreated with phenobarbital or Aroclor 1254, dosed with lindane, and fasted for 24 hr, the accelerated biotransformation of γ-HCH to chlorophenols may be more readily accommodated by enhanced glucuronidation than by an induced sulfotransferase system, which is saturated at a much lower dose than glucuronidation (36) and may be limited by the availability of inorganic sulfate (37). However, β-napthoflavone which did not significantly increase lindane metabolism (Table 3) also increased the glucuronides at the expense of the sulfates. This appears to be an example of either selective induction of the glucuronyl–transferase enzyme system (38) or selective inhibition of the sulfotransferase system (36).

Significant alterations in the urinary excretion of the individual lindane metabolites as free, glucuronide, or sulfate conjugates are presented in Tables 7–9. In general, phenobarbital and Aroclor 1254 pretreatment produced significant decreases in the proportion of the trichlorophenols excreted free or as sulfates but did not signifi-

[4] Abbreviations used: C, untreated control; LC, lindane control; N, β-naphthoflavone; P, phenobarbital; A, Aroclor 1254; dpm, disintegrations per minute; 2,4,6-TCP, 2,4,6-trichlorophenol; 2,3,5-TCP, 2,3,5-trichlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol; 2,3,4,6-TTCP, 2,3,4,6-tetrachlorophenol; 2,3,4,5-TTCP, 2,3,4,5-tetrachlorophenol; 2,3,4,6-TCCOL, 2,3,4,6-tetrachloro-2-cyclohexen-1-ol; γ-HCH, γ-hexachlorocyclohexane (lindane); PCCOL, 2,3,4,5,6-pentachioro-2-cyclohexen-1-o1; 2,4,5,6-TCCOL, 2,4,5,6-tetrachloro-2-cyclohexen-1-o1; γ-3,4,5,6-TCCH, γ-3,4,5,6-tetrachlorocyclohex-1-ene; γ-HCCH, γ-1,2,3,4,5,6-hexachlorocyclohexene; PCCH, 2,3,4,5,6-pentachlorocyclohexene; TCPMA, trichlorophenylmercapturic acid; DCPMA, dichlorophenylmercapturic acid; CPMA, chlorophenylmercapturic acid; PLP, microsomal phospholipid; PC, phosphatidylcholine; SP, sphingomyelin.

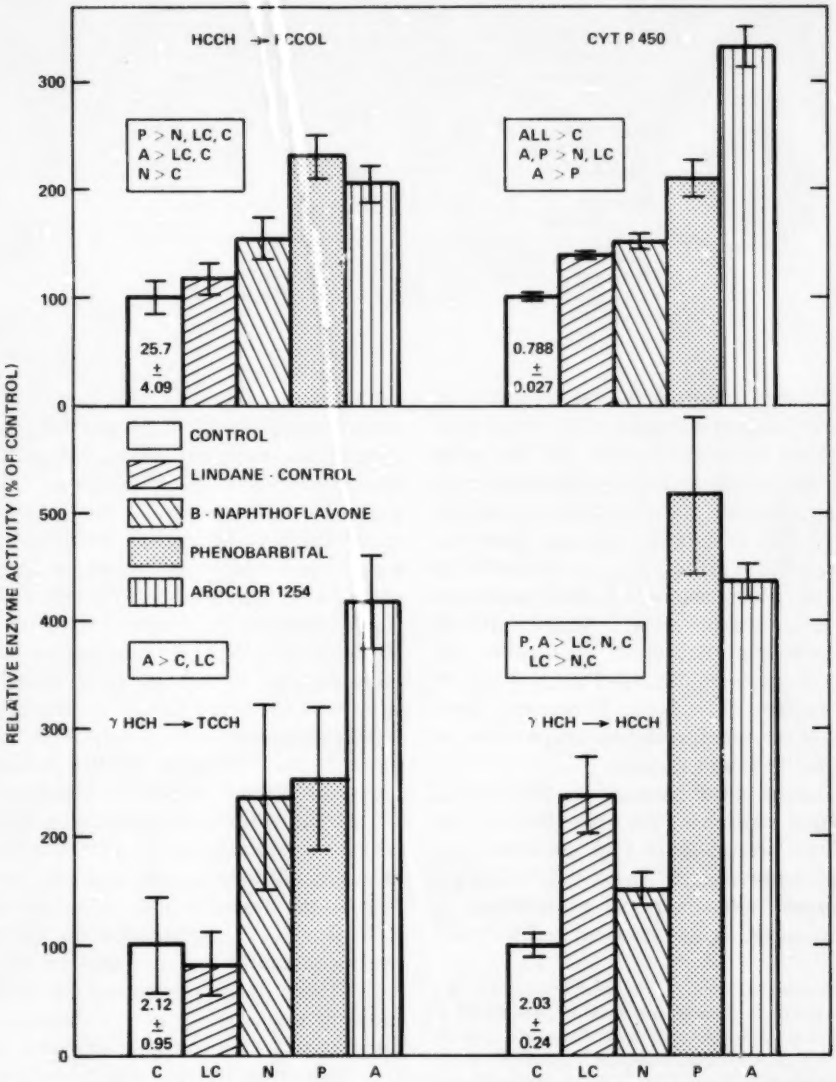

FIG. 1. *Effect of induction on the dehydrogenation of lindane (γ-HCH → HCCH; μg HCCH/g liver/30 min), the dechlorination of lindane (γ-HCH → TCCH; μg TCCH/g liver/30 min), the hydroxylation of the lindane intermediate, HCCH (HCCH → PCCOL; μg PCCOL/g liver/30 min), and the hepatic cytochrome P-450 content (nmol cytochrome P-450/mg microsomal protein). The mean value ± SE of the controls is recorded in the first bar for each response. These mean values are represented as 100% and the mean values of the other rats relative to the control are presented as percentage ± SE (treatment mean × 100/control mean). Vertical lines indicate the SE.*

cantly alter the glucuronide fraction. In contrast, they significantly reduced the proportion of the alcohols (2,3,4,6-TCCOL and PCCOL) excreted free or as glucuronides but did not affect the sulfates. Both treatments significantly increased the proportion of tetrachlorophenols in all fractions but Aroclor 1254 had a significantly

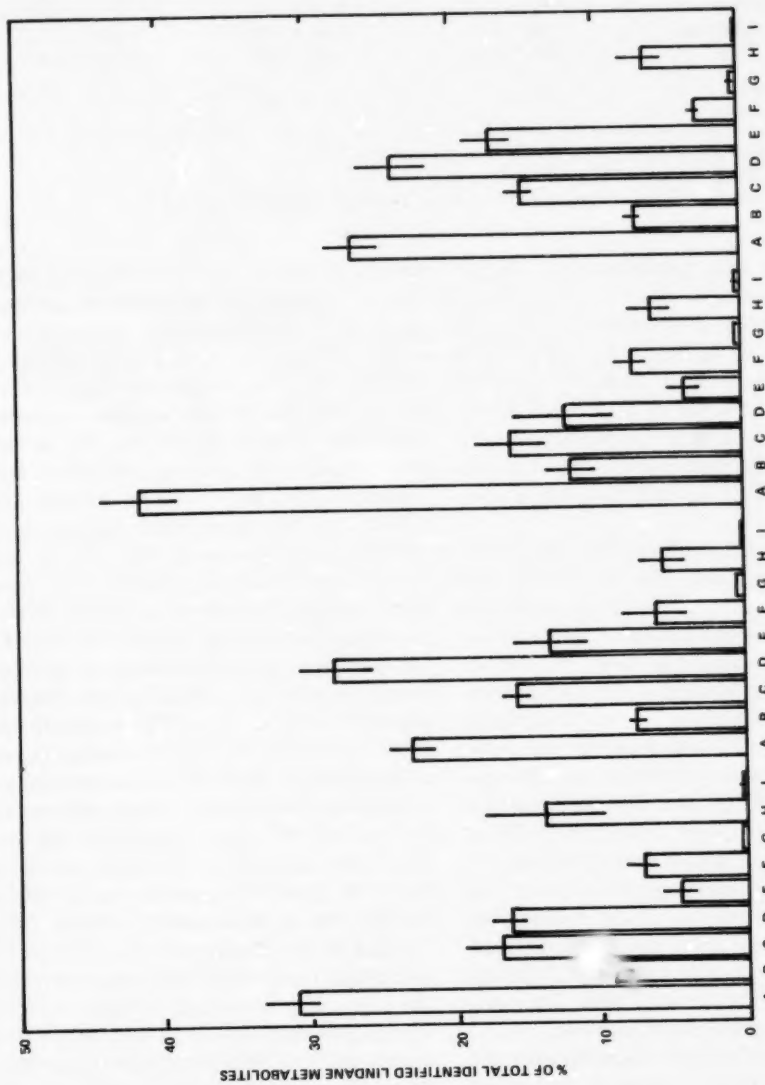

FIG. 2. *Effect of induction on the excretion pattern of eight lindane metabolites: A, 2,4,6-TCP; B, 2,3,5-TCP; C, 2,4,5-TCP; D, 2,3,4,6-TTCP; E, 2,3,4,5-TTCP; F, 2,3,4,6-TCCOL; G, 2,4,5,6-TCCOL; H, PCCOL; I, γHCH. The excretion pattern consists of the relative proportion of each metabolite from the total excretion of all eight metabolites plus unaltered lindane. Each bar is the mean of six animals. The vertical lines indicate the SE.*

TABLE 6
Effect of Induction on Conjugation of Identified Metabolites[a]

Treatment group[b]	Unconjugated	Conjugated sulfates	Conjugated glucuronides
1. Lindane control	5.53 ± 2.01	24.6 ± 4.21	69.9 ± 5.03
2. Phenobarbital	8.92 ± 2.52	8.49 ± 1.49	82.6 ± 3.18
3. β-Naphthoflavone	3.84 ± 1.47	11.6 ± 3.02	84.6 ± 4.22
4. Aroclor 1254	9.02 ± 1.10	4.69 ± 0.47	86.3 ± 0.87
Treatment comparisons[c]	N.S.	1 > All	All > 1

[a] Values are mean percentage of identified urinary metabolites ± SE.
[b] Treatment is described under Materials and Methods.
[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

greater effect than phenobarbital on the proportion excreted free or as sulfates. β-Naphthoflavone pretreatment resulted in the excretion of significantly higher proportions of unaltered lindane, and the glucuronides of 2,4,6- and 2,3,5-TCP. β-Napthoflavone treatment also significantly reduced the proportion of free 2,3,4,6-TTCP and PCCOL glucuronide below control levels.

Some significant alterations in microsomal phospholipid, the molar ratio of phosphatidyl choline to sphingomyelin and the molar ratio of cholesterol to phospholipid are presented in Table 10. Aroclor 1254 significantly altered all three responses, phenobarbital altered two, while β-naphthoflavone significantly changed only the molar ratio of phosphatidylcholine to sphingomyelin. It is well known that the induction of microsomal enzymes is associated with an increase in microsomal phospholipids (39, 40) and the apparent microviscosity of the lipid bilayer from biological membranes varies inversely with the phospholipid/cholesterol and phosphatidyl choline/sphingomyelin molar ratios (41). Thus pretreatment with phenobarbital, Aroclor 1254, and β-naphthoflavone, significantly increased membrane fluidity in this study (Table 10).

DISCUSSION

In the present study we have attempted to characterize changes in the normal metabolic fate of lindane following induction of microsomal enzymes by pretreatment with phenobarbital, β-naphthoflavone, or Aroclor 1254. Phenobarbital induces a set of cytochromes which exhibit maxima in the reduced carbon monoxide difference spectra at 450 nm (3), β-naphthoflavone induces cytochromes with maxima at 448 nm (42), and Aroclor 1254 induces cytochromes with maxima in the CO difference spectra at 448–449 nm (43). These hemoprotein cytochromes represent multiple species of a terminal electron transport oxidase which is involved in the hepatic biotransformation of xenobiotics such as lindane. Study of the chemical modification of NADPH–cytochrome *P*-450 reductase (44) in microsomes as well as temperature-dependent measurements of microsomal membrane bound enzyme activities (45–47) have supported the proposal that cytochrome proteins can be reduced by NADPH–cytochrome *P*-450 reductase due to their lateral mobility. Thus changes in the fluidity of the lipid in the microsomal membrane can potentially alter the rates of microsomal enzyme activity. Mammalian metabolism of lindane involves dechlorination, dehydrochlorination, dehydrogenation, glucuronyl transferase, sulfotransferase, glutathione *S*-transferase, hydroxylation via superoxide anion, and a variety of mixed-function oxidases such as that which initiates the direct hydroxylation of lindane to the intermediate penta-

TABLE 7

Effect of Induction on Excretion of Identified Free Metabolites[a]

Treatment group[b]	Percentage of total conjugated lindane metabolites						
	2,4,6-TCP[c]	2,3,5-TCP	2,4,5-TCP	2,3,4,6-TTCP	2,3,4,5-TTCP	2,3,4,6-TCCOL	γ-HCH
1. Lindane control	29.5 ± 3.57	10.6 ± 0.66	14.8 ± 1.33	22.6 ± 1.76	10.2 ± 1.89	3.15 ± 0.42	6.82 ± 1.23
2. Phenobarbital	18.8 ± 2.77	6.49 ± 0.90	10.6 ± 1.69	38.4 ± 1.06	16.7 ± 1.54	2.15 ± 1.16	0.69 ± 0.30
3. β-Naphthoflavone	28.9 ± 2.48	10.1 ± 0.79	12.3 ± 1.30	17.8 ± 0.73	11.4 ± 0.85	5.71 ± 1.21	12.2 ± 3.20
4. Aroclor 1254	22.3 ± 1.65	8.55 ± 0.44	5.09 ± 0.12	44.0 ± 1.08	16.0 ± 1.03	0.75 ± 0.24	0.37 ± 0.03
Treatment comparisons[d]	1,3 > 2	1,3,4 > 2	All > 4 1 > 2	4 > All 2,1 > 3 2 > 1	2,4 > 3,1	3 > 2,4	3 > All 1 > 2,4

[a] Values are mean percentage of total unconjugated lindane metabolites ± SE.

[b] Treatment is described under Materials and Methods.

[c] Abbreviations are defined in Footnote 4.

[d] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

chlorocyclohexenone-gem-chlorohydrin (9, 10, 27, 33, 35, 48–50) (Fig. 3).

Results from this study suggest that pretreatment with phenobarbital, Aroclor 1254, or β-naphthoflavone significantly altered the excretion pattern of lindane and its metabolites through rather selective effects on the multiple pathways involved in the biotransformation of the insecticide. A summary of the statistically significant responses are presented in Table 11. Of the 44 responses, Aroclor 1254, phenobarbital, and β-naphthoflavone pretreatments produced significant changes in 35, 32, and 18, respectively. Pretreatment with Aroclor 1254 or phenobarbital differed from pretreatment with β-naphthoflavone in that the former produced accelerated metabolism (Table 3) and stimulated enzyme activity (Fig. 1) whereas the latter did not significantly increase the rate of metabolism, selectively increased the hydroxylation of HCCH, impaired the dehydrogenation of lindane, and did not influence dechlorination.

However, though pretreatment with either phenobarbital or Aroclor 1254 produced a nearly identical fourfold increase in lindane metabolism (Table 3), there were important distinctions in the metabolite excretion patterns that were not consistent with equipotent, similarly acting enzyme inducers. For instance, rats pretreated with Aroclor 1254 expired threefold more lindane derived radioactivity (Table 3), and excreted a significantly higher proportion of neutral-lindane metabolites and a significantly lower proportion of polar-lindane metabolites than phenobarbital-dosed animals (Table 4). This difference may be due to the fact that only induction by Aroclor 1254 produced a significant increase in the anaerobic dechlorination of lindane to γ-3,4,5,6-TCCH (Fig. 1, Table 11), which is thought to be an intermediate in the metabolism of lindane to CO_2 (23, 51). Enhanced dechlorination of lindane may also account for the increased proportion of neutral metabolites in the urine of rats pre-

TABLE 8
Effect of Induction on Excretion of Identified Sulfates[a]

Treatment group[b]	Percentage of total sulfate-conjugated lindane metabolites				
	2,4,6-TCP[c]	2,3,5-TCP	2,4,5-TCP	2,3,4,6-TTCP	2,3,4,5-TTCP
1. Lindane control	46.0 ± 10.6	8.31 ± 3.90	20.7 ± 8.86	22.4 ± 2.39	0.90 ± 0.14
2. Phenobarbital	31.7 ± 3.33	2.29 ± 0.17	6.74 ± 0.44	52.6 ± 3.64	2.66 ± 0.87
3. β-Naphthoflavone	59.0 ± 2.46	4.36 ± 0.53	9.21 ± 0.68	21.8 ± 2.75	1.12 ± 0.19
4. Aroclor 1254	24.2 ± 0.69	2.35 ± 0.25	4.32 ± 0.26	62.0 ± 1.39	4.20 ± 0.82
Treatment comparisons[d]	3 > 2,4 1 > 4	1 > 4,2	1 > 2,4	4 > All 2 > 1,3	4 > 3,1

[a] Values are mean percentage of total sulfate-conjugated lindane metabolites ± SE.
[b] Treatment is described under Materials and Methods.
[c] Abbreviations are defined in Footnote 4.
[d] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

treated with Aroclor 1254 (Table 4). In addition, Aroclor 1254 pretreatment significantly increased the proportion of free and sulfate conjugated 2,3,4,6-TTCP above that excreted by rats pretreated with phenobarbital (Tables 7, 8). Aroclor 1254 pretreatment also enhanced the excreted proportion of sulfate-conjugated 2,3,4,5-TTCP (Table 8), and significantly decreased the excreted proportions of free 2,4,5-TCP (Table 7) and glucuronide-conjugated 2,3,4,6-TCCOL (Table 9) while phenobarbital pretreatment produced no significant effect. On the other hand, phenobarbital-treated rats excreted a significantly reduced proportion of unconjugated 2,3,5-TCP than the other animals. The significance of these changes in the metabolite profile are not apparent but may be related to differences in the liver to body weight ratio (Table 2), altered hepatic cytochrome *P*-450 content (Fig. 1, Table 11), and altered hepatic microsomal phospholipid content (Table 10). Hepatic microsomes from β-naphthoflavone-, phenobarbital-, and Aroclor 1254-pretreated rats had progressively increasing cytochrome *P*-450, total phospholipid, PC/SP molar ratios, and phospholipid/cholesterol molar ratios. These changes appear to be associated with observed differences in enzyme activity and lindane metabolism.

Unlike either phenobarbital or Aroclor 1254, β-naphthoflavone failed to induce significantly higher lindane metabolism (Table 3). Nevertheless, β-naphthoflavone pretreatment significantly increased the proportion of glucuronide conjugates at the expense of the sulfates (Table 6) and produced a significant departure from the control excretion pattern for eight lindane metabolites (Fig. 2). Significantly increased proportions of unaltered lindane, 2,4,6-TCP and 2,3,5-TCP were excreted by rats dosed with β-naphthoflavone (Fig. 2). Lindane is metabolized to 2,3,4,6-tetrachlorphenol by a pathway distinct from that leading to 2,4,6-trichlorophenol (Fig. 3). The two competing pathways respond differently to induction, with metabolism through γ-HCCH to 2,3,4,6-TTCP being the more sensitive (10, 52). Induction of the dehydrogenation of lindane to γ-HCCH was stimulated by phenobarbital and Aroclor 1254 but impaired by β-naphthoflavone (Fig. 1). As a result, phenobarbital- and Aroclor 1254-pretreated rats excreted significantly greater proportions of 2,3,4,6-TTCP whereas β-naphthoflavone-dosed rats excreted a significantly greater proportion of 2,4,6-TCP (Fig. 2). Also as a result of the impaired dehydrogenase activity noted above, β-naphthoflavone pretreatment significantly reduced the proportions of free 2,3,4,6-TTCP (Table 7) and glucuronide-conjugated PCCOL (Table 9) be-

TABLE 9

Effect of Induction of Excretion of Identified Glucuronides[a]

Treatment group[b]	Percentage of total glucuronide-conjugated lindane metabolites					
	2,4,6-TCP[c]	2,3,5-TCP	2,3,4,6-TTCP	2,3,4,5-TTCP	2,3,4,6-TCCOL	PCCOL
1. Lindane control	25.9 ± 2.99	8.95 ± 0.81	13.5 ± 1.85	5.38 ± 1.34	9.74 ± 1.64	21.3 ± 8.10
2. Phenobarbital	22.3 ± 1.42	7.94 ± 0.50	24.5 ± 2.68	14.2 ± 2.60	7.17 ± 2.48	6.19 ± 1.70
3. β-Naphthoflavone	38.8 ± 3.37	12.8 ± 1.66	10.9 ± 3.45	4.41 ± 1.28	8.67 ± 1.32	7.50 ± 1.78
4. Aroclor 1254	27.8 ± 2.12	7.37 ± 0.51	19.8 ± 2.53	18.0 ± 2.13	3.33 ± 0.45	7.15 ± 1.66
Treatment comparisons[d]	3 > All	3 > All	2 > 1,3 4 > 3	4,2 > 1,3	1,3 > 4	1 > All

[a] Values are mean percentage of total glucuronide-conjugated lindane metabolites ± SE.
[b] Treatment is described under Materials and Methods.
[c] Abbreviations are defined in Footnote 4.
[d] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

TABLE 10

Effect of Pretreatment on Cholesterol, Sphingomyelin, and Microsomal Phospholipid

Treatment group[a]	Total phospholipid[b]	PC/SP ratio[c]	Phospholipid/cholesterol[d]
1. Controls	7.20 ± 0.449	30.0 ± 1.82	8.21 ± 0.590
2. Lindane controls	7.30 ± 0.346	34.6 ± 1.26	10.5 ± 1.07
3. Phenobarbital	9.19 ± 0.863	41.9 ± 2.06	10.6 ± 0.69
4. β-Naphthoflavone	7.89 ± 0.319	39.4 ± 2.93	10.1 ± 0.95
5. Aroclor 1254	11.3 ± 0.721	80.2 ± 1.68	13.6 ± 0.88
Treatment comparisons[e]	5 > All 3 > 2,1	5 > All 3,4 > 1 3 > 2	5 > All

[a] Treatment is described under Materials and Methods.
[b] Values are mean μm phospholipid/g liver.
[c] Values are mean molar ratios of phosphatidyl choline/sphingomyelin in microsomal preparations.
[d] Values are mean molar ratios of phospholipid/cholesterol in microsomal preparations.
[e] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test.

TABLE II

Summary of Significant Responses to Pretreatment

Response[a,b]	Comp.[c]	Response	Comp.	Response[d]	Comp.	Response[d]	Comp.
Total excreted	5,3 > 4,2	Liver wt/body wt	All > 1 5 > 3,4,2	Free 2,4,6-TCP	2,4 > 3	Gluc. 2,4,6-TCP	4 > 5,2,3
Urinary	5,3 > 4,2	Cytochrome *P*-450	All > 1 5,3 > 4,2 5 > 3	Free 2,3,5-TCP	2,4,5 > 3	Gluc. 2,3,5-TCP	4 > 2,3,5
Fecal	3 > 4	Dehydrogenase	3,5 > 2,4,1 2 > 1	Free 2,4,5-TCP	2,4,3 > 5	Gluc. 2,3,4,6-TTCP	3 > 2,4 5 > 4
Expired	5 > 3,4,2	Dechlorinase	5 > 1,2	Free 2,3,4,6-TTCP	5 > 3,2,4 3,2 > 4 3 > 2	Gluc. 2,3,4,5-TTCP	5,3 > 2,4
Urinary neutrals	5,3 > 4,2	HCCH hydroxylation	3,5 > 2,1 3 > 4 4 > 1	Free 2,3,4,5-TTCP	3,5 > 4,2	Gluc. 2,3,4,6-TTCOL	2,4 > 5
Urinary FCP	5,3 > 4,2	Total phospholipid	5 > All 3 > 2,1	Free 2,3,4,6-TCCOL	4 > 3,5	Gluc. PCCOL	2 > 4,5,3
Urinary CCP	3,5 > 2,4	PC/SP molar ratio	5 > All 3,4 > 1 3 > 2	Sulfate 2,4,6-TCP	4 > 3,5 2 > 5	Total 2,4,6-TCP	4 > 2,5,3
Urinary polar	3,5 > 4,2	PLP/chol. molar ratio	5 > All	Sulfate 2,3,5-TCP	2 > 5,3	Total 2,3,5-TCP	4 > 3,5
Liver	4,2 > 3,5	% Unaltered γ HCH	4 > 2,3,5 2 > 3,5	Sulfate 2,4,5-TCP	2 > 3,5	Total 2,3,4,6-TTCP	3 > 2,4 5 > 4
Kidney	4,2 > 5,3	% Sulfates	2 > 4,3,5	Sulfate 2,3,4,6-TTCP	5 ± 3,2,4 3 > 2,4	Total 2,3,4,5-TTCP	5,3 > 2,4
Adipose	4,2 > 5,3	% Glucuronides	5,4,3 > 2	Sulfate 2,3,4,5-TTCP	5 > 4,2	Total PCCOL	2 > 5,4,3

[a] Abbreviations are defined in Footnote 4 in text.

[b] Responses were expressed as dpm.

[c] Comparisons are significantly different at $P < 0.05$ by Duncan's multiple range test with the following treatment designations: (1) untreated controls; (2) rats not pretreated that received lindane 24 hr before sacrifice; (3) phenobarbital pretreated rats; (4) β-naphthoflavone pretreated rats; (5) aroclor 1254 pretreated rats. Treatments are described under Materials and Methods.

[d] Responses were expressed as percentages.

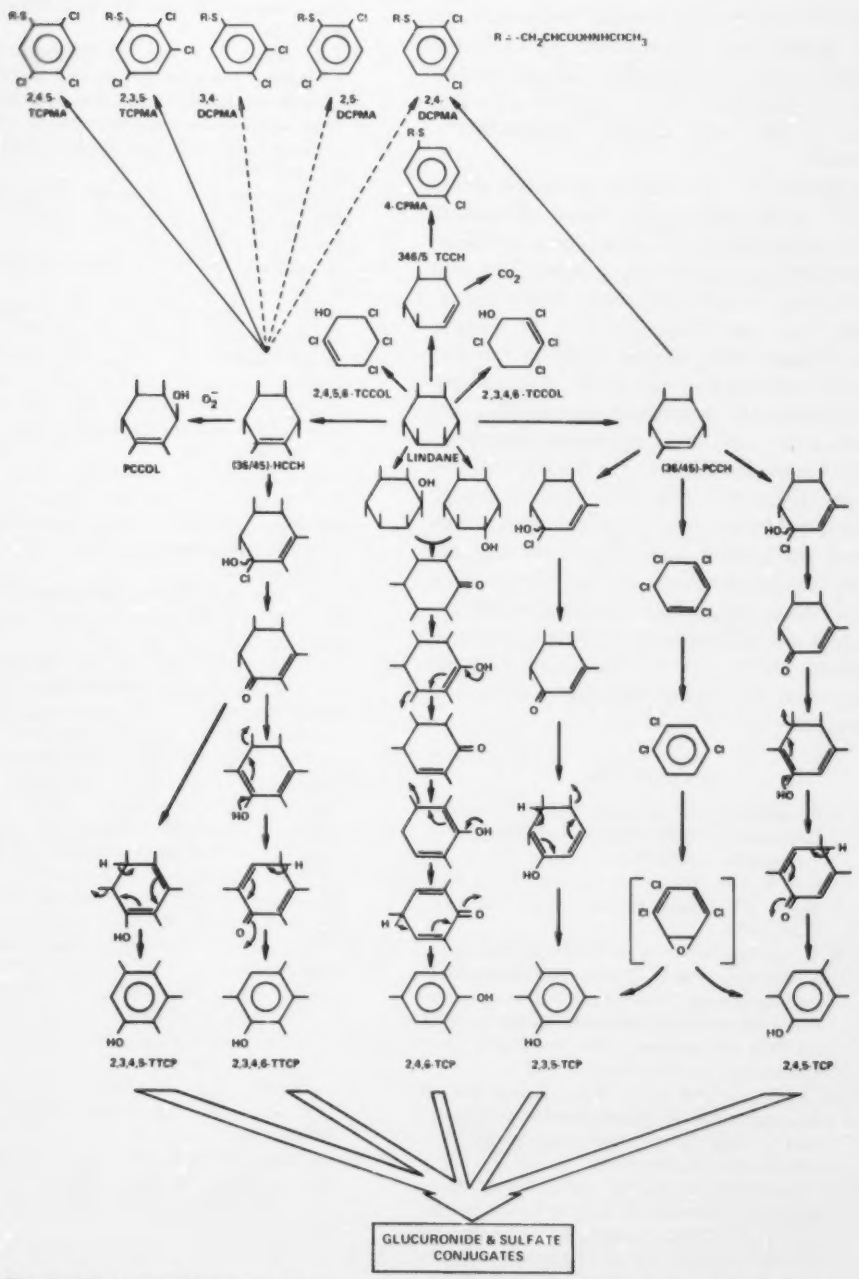

FIG. 3. *The biotransformation of lindane in rats. Where the configuration of the metabolites is known, this is indicated by a fractional notation, numbers in the numerator denoting chlorine above and numbers in the denominator denoting chlorine below the plane of the ring. Abbreviations are defined in Footnote 4.*

low control levels. Similar reductions in the proportion of excreted PCCOL by phenobarbital- and Aroclor 1254-pretreated rats, on the other hand, indicates a preference for the route leading to tetrachlorophenols.

Significant and rather selective alterations in the biotransformation of lindane were observed in this study after pretreatment with phenobarbital, Aroclor 1254, and even after β-naphthoflavone, which apparently does not affect the rate of lindane metabolism when administered in the diet at the level used in this study. Since many toxicologically hazardous metabolites are formed in small or even minute quantities (53), it appears feasible, on the basis of this work, that preferential induction of an activation pathway could lead to toxicity even when the overall rate of toxicant metabolism remains unchanged. This observation could have important implications in exposure assessment where multiple environmental chemicals are involved and the potential for toxic interactions is appreciable.

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